

Sexually-transmitted diseases in women

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Most acute or recurrent genital infections are caused by micro-organisms other than *N. gonorrhoeae* (Willcox, 1972). Recently, the possible significance of mycoplasmas in genital infections has especially been studied. *M. hominis* is commonly isolated from patients with cervicitis, vaginitis, or pelvic inflammatory disease (McCormack, Braun, Lee, Klein, and Kass, 1973). Two cases of T-mycoplasmaemia have been reported in pregnant women (Sompolinsky, Solomon, Leiba, Caspi, Lewinsohn, and Almog, 1971; Caspi, Herczeg, Solomon, and Sompolinsky, 1971), and it has been suggested that T-mycoplasmas may be responsible for some cases of spontaneous abortion (Caspi, Solomon, and Sompolinsky, 1972).

Group B streptococci in the vagina during pregnancy are considered to be a potential source of neonatal sepsis (Franciosi, Knostman, and Zimmerman, 1973). *Trichomonas vaginalis* and *Candida albicans* are generally regarded as vaginal pathogens, as is also *Corynebacterium vaginale* (*Haemophilus vaginalis*) (Gardner and Dukes, 1955). Vulvitis and vaginitis are frequently associated with *Trichomonas* and *Candida* but not with *C. vaginale* (Robinson and Mirchandani, 1965). *Candida* is said to be influenced by the female menstrual and pregnancy cycles (Drake and Maibach, 1973).

We here describe a study planned to evaluate the relationship between the cervical microbiology and the presence of symptoms or signs of venereal disease in a series of consecutive patients at the time of their first visit to the clinic.

Material and methods

PATIENTS

All new female patients attending the Venereal Disease Clinic of the University Hospital in Uppsala between February and May, 1972, were included in this study. Excepted were patients who had taken antibiotics during

a 2-week period before their first visit and patients already treated elsewhere who were seen only for follow-up.

The final series comprised 341 women ranging in age from 14 to 40 years (mean 22).

METHODS

A careful history was taken from each patient. The data were coded for computer analysis, as were those obtained by clinical examination and microbiological cultures. 54 per cent. of the patients were examined by Dr. Wallin and the remainder by other venereologists working at the clinic.

CLINICAL PROCEDURE

Four specimens were taken from the cervical canal with charcoal-coated cotton-wool swabs and placed in separate tubes of transport medium:

- (1) For bacteria, trypticase soy broth (Difco) partly stabilized with 3 per cent. sucrose ;
- (2) For 'classical' mycoplasmas, mycoplasma broth (Chanock, Hayflick, and Barile, 1962);
- (3) For T-mycoplasmas, Shepard's U9 broth (Shepard and Lunceford, 1970);
- (4) For protozoa and yeasts, Diamond's medium (Diamond, 1957).

Slides were made from the first specimen and stained with methylene blue for direct microscopical examination for polymorphonuclear leucocytes. Ten or more polymorphonuclear leucocytes per high-power field (HPF) seen in several fields using a $\times 100$ objective and $\times 12.5$ eyepiece were recorded as a leucocytic reaction; fewer than ten WBC/HPF were recorded as no reaction.

Fresh smears of vaginal discharge in saline were examined for *T. vaginalis* within 5 minutes after collection, as recommended by Eddie (1968).

The criteria used for classification of the patients were whether they complained of vaginal discharge and the number of leucocytes detected by direct microscopical examination of smears from the cervix. A vaginal discharge of more than 1 year's duration and which was considered normal by the patient was not recorded.

MICROBIOLOGICAL PROCEDURE

The time taken for transportation of the specimens to the laboratory never exceeded 5 hrs. On arrival at the

laboratory, material from the swabs was inoculated onto conventional and protective media for the isolation of *N. gonorrhoeae* and L-phase organisms as described earlier (Gnarpe, Wallin, and Forsgren, 1972). Standard laboratory media (blood agar base plates—Difco, sodium-azide agar—Difco, haematin agar plates and CLED agar plates—Baltimore Biological Laboratory) were also inoculated for determination of the bacterial flora. All plates were incubated at 37°C. for 48 hrs. The different bacterial species were indentified by means of standard laboratory techniques.

The swabs taken for mycoplasma isolation were transferred to fresh mycoplasma broth (Chanock, Hayflick and Barile, 1962) and to fresh U9 broth (Shepard and Lunceford, 1970). The conditions of cultivation were essentially those previously described (Gnarpe and Friberg, 1972).

'Classical' mycoplasmas were typed according to Clyde (1964) or, if they were not *M. hominis*, according to Aluotto, Wittler, Williams, and Faber (1970). T-mycoplasmas were identified by their capacity to split urea, their typical colonial morphology, and their sensitivity to erythromycin.

Trichomonas vaginalis was cultivated as described earlier (Wallin and Forsgren, 1974). Subcultures were also made from *Trichomonas* media onto Sabouraud's medium (Difco) for isolation of yeasts. *Candida albicans* was distinguished from other yeasts by the use of Dubos' medium, but no further typing was done.

Results

The frequencies of different micro-organisms in cultures from cervical specimens are shown in Table I.

TABLE I *Microbiological findings in cervical specimens from 341 women*

Organisms	Type	Percentage
Bacteria	<i>Staph. aureus</i>	3
	<i>Staph. albus</i>	44
	Streptococci	11
	Enterococci	10
	<i>N. gonorrhoeae</i>	20
	<i>C. vaginale</i>	3
	L-forms	13
Mycoplasmas	<i>M. hominis</i>	32
	T-mycoplasmas	53
Yeasts	<i>C. albicans</i>	28
	Others	6
Protozoa	<i>T. vaginalis</i>	12

In Tables II to V, the occurrence of the most common micro-organisms has been correlated with various criteria. *Staphylococcus albus* and the organisms occurring in low frequency shown in Table I are not included here, but they were evenly distributed among the various groups.

The correlation between culture results and the leucocytic reaction seen on direct microscopical examination of the smears is shown in Table II.

TABLE II *Percentage of positive findings in cervical specimens from women with and without leucocytic reaction in the cervical smear*

Type of culture	Percentage of positive cultures	
	97 women with leucocytic reaction	244 women without leucocytic reaction
<i>N. gonorrhoeae</i>	36 ^a	13
<i>M. hominis</i>	27	35 ^a
T-mycoplasmas	61	51
<i>C. albicans</i>	25	29
<i>T. vaginalis</i>	20 ^b	9

^aP < 0.001

^bP < 0.01

36 per cent. of the women with a leucocytic reaction in the cervical smear had positive cultures for *N. gonorrhoeae*, compared with 13 per cent. of the women without a leucocytic reaction ($P < 0.001$); *T. vaginalis* was also more common among those with a leucocytic reaction ($P < 0.01$); the corresponding figures were 20 and 9 per cent. respectively.

The micro-organisms isolated from 67 women with a gonococcal infection are shown in Table III, in which the patients are divided into groups according to leucocytic reaction in the cervical smear and symptoms. A leucocytic reaction was found in 35 women (52 per cent.), and forty women (60 per cent.) complained of vaginal discharge. 40 per cent. of the patients with a leucocytic reaction had positive cultures for *M. hominis* and 20 per cent. for *T. vaginalis*. The corresponding figures for those without a leucocytic reaction were 25 and 6 per cent. respectively. These differences, however, are not significant. The percentages of positive cultures for all non-gonococcal micro-organisms were about the same among women with and without symptoms.

TABLE III *Findings in cervical specimens from 67 women with gonococcal infection. Patients grouped according to leucocytic reaction and symptoms*

Type of culture	Leucocytic reaction (per cent.)	No leucocytic reaction (per cent.)	Vaginal discharge (per cent.)	No vaginal discharge (per cent.)
<i>M. hominis</i>	40	25	30	37
T-mycoplasmas	66	69	72	63
<i>C. albicans</i>	20	19	18	22
<i>T. vaginalis</i>	20	6	13	15
Total number of patients	35	32	40	27

In Table IV, the culture findings from cervical specimens of women with *N. gonorrhoeae*, *T. vaginalis*, and *M. hominis* are compared with the findings from a control group of patients without gonorrhoea and without signs of venereal disease, i.e. no discharge and no leucocytic reaction in the smear. The control group had fewer *T. mycoplasmas* ($P < 0.01$) than the other groups. Unspecified L-phase organisms were found in 28 per cent. of the women with a growth of *T. vaginalis*, a significant difference from the 6 per cent. found in the control group ($P < 0.001$).

Positive cultures for *C. albicans* were found to increase in frequency during the later phases of the menstrual cycle, as demonstrated in Table V. In this Table the mid-cycle represents a period of 2 weeks and the other parts of the cycle 1 week each.

Contraceptive pills were used by 54 per cent. of the women and there were no microbiological differences between users and non-users.

TABLE V Microbiological findings in specimens from 341 women correlated with phases of the menstrual cycle

Type of culture	Days of the menstrual cycle (per cent.)				Total
	0-7	8-21	22-30	>30	
<i>N. gonorrhoeae</i>	19	51	21	9	100
L-forms	20	68	9	3	100
<i>M. hominis</i>	17	55	20	8	100
<i>T. mycoplasmas</i>	18	51	23	8	100
<i>C. albicans</i>	11	52	31	6	100
<i>T. vaginalis</i>	18	55	20	7	100
Total percentage of patients	17	54	23	6	100

Table VI compares the results of direct microscopical examination of smears for *T. vaginalis* with the results of cultures. Among 42 women harbouring *T. vaginalis*, the parasite could be demonstrated in

wet smears in 61 per cent. and culture was negative in only one case. Positive smears as well as cultures were found, however, in 86 per cent. of those women who had clinical symptoms of *T. vaginalis* infection.

TABLE VI Correlation of *T. vaginalis* diagnosed by vaginal smear and by cervical culture in 42 women

Culture	Positive		Negative
	Smear	Culture	
Smear	Positive	25	1
	Negative	16	-

Various methods of detecting *T. vaginalis* and transporting specimens to the laboratory were used from February to September, 1972. In 324 cases one charcoal-coated swab was taken from the cervical canal and another from the vaginal fornix and both were sent to the laboratory in Diamond's medium. Positive cultures were obtained from 45 and 44 respectively. During another period, 954 duplicate cervical swabs were taken and sent in Stuart's and Diamond's media. The time taken in transit did not exceed 5 hrs. Positive cultures resulted from 86 (9 per cent.) and 95 (10 per cent.) of these specimens respectively. Concomitant cultures for *N. gonorrhoeae* were inoculated from the swabs sent in Stuart's medium.

Discussion

Patients attending a clinic for venereal diseases present many different genital disorders, of which the 'non-specific' infections form the major part (Department of Health and Social Security, 1971). The results of this study indicate that most bacteria have no importance for the development of vaginal discharge or of a cervical reaction with an increased number of leucocytes.

TABLE IV Percentages of positive cultures from cervical specimens grouped according to microbiological criteria

Type of culture	Positive cultures			Negative <i>N. gonorrhoeae</i> No discharge or leucocytic reaction (per cent.)
	<i>N. gonorrhoeae</i> (per cent.)	<i>M. hominis</i> (per cent.)	<i>T. vaginalis</i> (per cent.)	
<i>N. gonorrhoeae</i>	100	25	23	0
L-forms	9	15	28 ^a	6
<i>M. hominis</i>	33	100	48	28
<i>T. mycoplasmas</i>	67 ^b	69 ^b	73 ^a	45
<i>C. albicans</i>	19	21	15	28
<i>T. vaginalis</i>	13	17	100	6
Total no. of patients	67	111	41	97

^a $P < 0.001$ | for groups with positive cultures compared with group negative

^b $P < 0.01$ | for *N. gonorrhoeae* and without discharge or leucocytic reaction

Streptococci were cultured from 11 per cent. of the women, but Group B was found in only two cases. Other investigators (Gardner and Dukes, 1955) have reported a higher incidence of *C. vaginale* than we found. This may be because we did not use a special medium for the cultivation of *C. vaginale*.

M. hominis was isolated from 32 per cent. of the patients. In a group of pregnant patients without symptoms, the corresponding figure was 13 per cent. (Gnarpe and Friberg, 1972; Gnarpe, 1973). The presence of *M. hominis* in cervical specimens, however, did not seem to be of importance for the development of symptoms, as it was isolated in similar numbers from women with and without symptoms and from women with and without a leucocytic reaction.

T-mycoplasmas were isolated from 67 per cent. of the patients with *N. gonorrhoeae*, compared with 45 per cent. of controls without signs of infection and 23 per cent. of pregnant women without symptoms (Gnarpe and Friberg, 1972). It is possible, however, that the relatively high frequency of T-mycoplasmas in the gonorrhoea group merely reflects sexual activity, as has been reported in a study by McCormack, Almeida, Bailey, Grady, and Lee (1972). We found that 13 per cent. of our controls had not had intercourse during the last 30 days, compared with 4 per cent. in the gonorrhoea group. This difference, however, is not statistically significant.

The increasing incidence of gonorrhoea is now recognized as being largely due to asymptomatic infection (Pariser, 1972), and this type of gonorrhoea is also responsible for many of the cases with disseminated infection, especially among women (Holmes, Counts, and Beaty, 1971). We found no significant differences in the microbiological findings between patients with symptomatic and asymptomatic gonorrhoea or between patients with and without a leucocytic reaction. *N. gonorrhoeae* and *T. vaginalis* were the only organisms isolated significantly more often from women with a leucocytic reaction. This is in agreement with our earlier findings (Wallin, 1974), using the leucocytic reaction as an indication of gonorrhoea. Therefore, failure to isolate *N. gonorrhoeae* in cases with a leucocytic reaction is suggestive of a *T. vaginalis* infection. Growth of L-forms occurs with a high frequency in patients infected with *T. vaginalis*. The relevance of this finding is, for the moment, unknown.

Recent reports have suggested a causative relationship between oral contraceptives and vaginal candidosis (Catterall, 1971). In this investigation we could find no difference in the frequency of vaginal candidosis between users and non-users. This is probably

due to the use of contraceptives with a low hormonal content. In most investigations in which *Candida* infection was positively correlated with the use of oral contraceptives, preparations with higher hormonal content were involved (Yaffee and Grots, 1965; Porter and Lyle, 1966; Catterall, 1966); the hormonal content has been shown to be of importance for the presence of *Candida* (Rohatiner and Grimble, 1970).

Trichomonas vaginalis was cultured from specimens from 41 women. An immediate diagnosis could be established by microscopical examination of unstained specimens in only 61 per cent. of these cases, which is in accordance with the findings of Bartunek and Schultze (1972). In symptomatic cases, however, *T. vaginalis* could be detected microscopically in 86 per cent. Because of the high frequency of *Trichomonas* infection in patients with a leucocytic reaction, culture for *T. vaginalis* is especially recommended for such patients in whom no organisms can be detected by direct microscopical examination of the wet smear. This investigation shows that specimens taken on charcoal-coated swabs and sent to the laboratory in Stuart's transport medium are as satisfactory for the culture of *T. vaginalis* as they were earlier demonstrated to be for *N. gonorrhoeae* (Gästrin and Kallings, 1968). Similar results were obtained when *T. vaginalis* was cultured from specimens taken at the same time from vagina and cervix and transported in Diamond's medium.

Summary

The cervical microbiology has been studied in 341 consecutive new patients attending a Venereal Disease Clinic. The results indicate that most bacteria have no influence on the development of vaginal discharge or on an increase in the number of leucocytes in the cervical secretion. Mycoplasmas were found significantly more often in patients with a gonococcal infection, but the presence of mycoplasmas could not be correlated with symptoms of genital infection or with a leucocytic reaction in the cervical material. Such a reaction was found significantly more often in patients with *N. gonorrhoeae* or *T. vaginalis*. Culturing for *T. vaginalis* is therefore especially recommended in patients with a leucocytic reaction in whom no gonococci or flagellates can be detected by direct microscopical examination of smears. For culture of *T. vaginalis*, cervical specimens taken with charcoal-coated cotton-wool swabs and transported in Stuart's medium gave results comparable with specimens taken from the vaginal secretion and transported in Diamond's medium.

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Les maladies sexuellement transmises chez la femme

SOMMAIRE

La microbiologie cervicale a été étudiée chez 341 nouvelles consultantes consécutives dans une clinique vénéréologique. Les résultats montrent que la plupart des bactéries n'ont pas de signification en ce qui regarde l'existence d'une sécrétion vaginale ou l'augmentation du nombre des leucocytes dans les prélèvements cervicaux. Des mycoplasmes furent trouvés significativement plus souvent chez les sujets atteints de gonococcie, mais la présence de mycoplasmes fut sans rapport avec les symptômes d'infection génitale ou avec une réaction leucocytaire dans les prélèvement cervical. Une telle réaction a été trouvée significativement plus fréquente chez les malades atteintes d'infection à *N. gonorrhoeae* ou à *T. vaginalis*. La culture pour *T. vaginalis* est donc spécialement recommandée chez les malades présentant une réaction leucocytaire et chez lesquelles l'examen microscopique des sécrétions ne montre ni gonocoques ni flagellés. Pour la culture de *T. vaginalis*, les spécimens cervicaux pris avec un écouvillon de coton charbonné et transporté en milieu de Stuart ont donné des résultats comparables à ceux des spécimens de sécrétions vaginales transportés en milieu de Diamond.